J. Physiol. (1983), **336**, *pp.* 131–142
Printed in Great Britain

GLUCOSE HANDLING BY DISTAL PORTIONS OF THE NEPHRON DURING PREGNANCY IN THE RAT

BY J. H. V. BISHOP† AND R. GREEN*

From the Department of Physiology, University of Manchester, Oxford Road, Manchester M13 9PT

(*Received 29 July 1982*)

SUMMARY

1. Reabsorption of glucose, salt and water was measured in 7–8 day pregnant and virgin rats by micropuncturing distal nephrons during saline and glucose infusions. Unidirectional fluxes of glucose were measured in loops of Henle and collecting ducts.

2. There were no significant differences in single nephron glomerular filtration rate (S.N.G.F.R.) between virgin and pregnant animals during saline infusion. During glucose infusion S.N.G.F.R. was higher in pregnant animals than in virgins.

3. There is no evidence of a failure of reabsorption of glucose by the proximal tubule in pregnant animals.

4. More glucose is reabsorbed from the loop of Henle in virgin animals than in pregnant animals during saline infusion but during glucose infusion the converse is true.

5. During both saline and glucose infusion there is less reabsorption of glucose from the collecting duct in pregnant animals than in virgin animals.

6. It is concluded that the increased excretion of glucose during pregnancy can be attributed to alteration of glucose handling by distal segments of the nephron.

INTRODUCTION

During pregnancy there are adaptive changes in many of the body's systems; some of the most dramatic of these are changes in renal function. Thus, there is an increase in glomerular filtration rate, an increased reabsorption of sodium and water (see Garland & Green, 1982 for references) and an increased glucose excretion. This increased glucose excretion is well documented in women (see Davison & Hytten, 1975 for references) and has recently also been shown to occur in rats (Bishop & Green, 1980).

It has long been assumed that all glucose reabsorption occurs in the first part of the proximal tubule, and so the cause of the increased urinary glucose excretion has been ascribed to either an increased filtered load of glucose (Christiansen, 1958) or to a decreased reabsorptive capacity of the proximal tubule (Welsh & Sims, 1960); in either case the glycosuria may be exacerbated by previously undetected renal disease

* To whom reprint requests and correspondence should be sent.
† Present address: 31 Bridgeview Road, Engadine, New South Wales 2233, Australia.

(Davison & Hytten, 1975). Direct evidence in the rat, obtained by micropuncture investigations of proximal tubular function, has indicated, however, that proximal tubular dysfunction is not the cause of the glycosuria of pregnancy (Bishop & Green, 1981) so other causes must be sought.

It is now well documented that glucose reabsorption occurs beyond the proximal convoluted tubule, occurring at least in the pars recta (von Baeyer, 1975), the loop of Henle (Bishop, Green & Thomas, 1981) and the collecting duct (Fröhnert, Hohmann, Zwiebel & Baumann, 1970). Under physiological conditions the amount of glucose presented to and reabsorbed by these segments is small, but it has been suggested that a decreased reabsorption by these later parts of the nephron is responsible for the increased glucose excretion during pregnancy (Bishop & Green, 1981).

This study was designed to investigate directly reabsorption of glucose from distal segments of the nephron under conditions of normal and elevated plasma glucose concentrations in virgin and pregnant rats. Some of the preliminary results have been published in abstract form (Bishop & Green, 1979*a*, *b*).

METHODS

All experiments were performed on either virgin or 7–8 day pregnant Sprague–Dawley rats 12–13 weeks old at the time of the experiments. Animals had access to a rat pellet diet (PMD, Na content 0·06 μmol/g^{-1}) until 16 h before the experiment, and water until the experiments began. Rats were anaesthetized with Inactin (5-ethyl-5-(1′-methyl-propyl)-2-thiobarbiturate; Inter Pharm, Hamburg) 120 mg kg body weight^{-1} I.P. and prepared for micropuncture as previously described (Bishop, Green & Thomas, 1978).

Saline (0·15 M) was given as a priming dose of 1 ml and then as a continuous infusion (200 μl min^{-1}) for 4 h; this was followed by an infusion of 5% D-glucose at the same rate for 3 h. The first 1½ h of saline infusion and the first 30 min of glucose infusion were allowed for stabilization of plasma and urinary concentrations of glucose (see Bishop & Green, 1980). Three types of experiments were performed.

Free flow distal micropuncture

For these experiments the infusates contained [^{3}H]inulin 5 μCi ml^{-1}. Distal tubules were identified and punctured as previously described (Garland & Green, 1982); pipettes with an outside diameter of 8–10 μm were used to collect samples for timed intervals, usually 8–10 min. Blood samples (50 μl) were collected from a tail vein hourly throughout the experiment.

The volume of fluid collected from each puncture was measured in a calibrated constant bore capillary (i.d. ~ 0·3 mm) and aliquots of collected tubular fluid and plasma analysed as follows: [^{3}H]inulin, using P.C.S. (Radiochemical Centre, Amersham) diluted 1:1 with A.R. Toluene as a scintillant, in a liquid scintillation counter; sodium and potassium concentrations on a Helium Glow Photometer (Aminco, Silver Springs, MD, U.S.A.); glucose concentration using an enzymatic method described previously (Bishop *et al.* 1978).

Calculations

Single nephron glomerular filtration rate	S.N.G.F.R. $= \dot{V}\ (TF_{in}/P_{in})$
Nephron filtered load	S.N.G.F.R. $\cdot P_a$
Fluid reabsorption	$\dot{V}\ ([(TF_{in}/P_{in})]^{-1})$
Solute reabsorption	S.N.G.F.R. $\cdot P_a - \dot{V}\ TF_a$
Percentage reabsorption	$100\ (1-[TF_a/P_a)]/[TF_{in}/P_{in})])$

where $\dot{V}$ is rate of fluid collection, TF and P refer to tubular fluid and plasma concentrations of inulin (in) or any another substance (a). Fourteen virgins and fourteen pregnant animals were used in this series.

Microinjection studies of loop of Henle

In animals prepared for micropuncture, short loops of Henle were selected as follows. Randomly selected proximal tubules were punctured with a sharpened micropipette (tip diameter 7 μm) which contained 1% Lissamine Green, and a small bolus of the dye was injected. This permitted identification of the last surface proximal convolution and, after a further 15–30 s, the first distal convolution on the kidney surface. When both of these sites were considered accessible to micropuncture a second pipette (tip diameter 10–12 μm) was inserted into the identified distal segment and a column of oil at least 5 × the tubular diameter was injected distally; collection of the fluid was then begun. A third pipette, manufactured with a tip diameter of 6 μm and a constriction so that it contained a constant volume of 5–10 nl, was inserted into the last proximal convolution. This pipette was filled with fluid containing NaCl, 150 mmol l^{-1}; KCl, 4 mmol l^{-1}; [^{3}H]inulin, 159 mCi l^{-1}, D-[^{14}C]-glucose, 178 mCi l^{-1}; which was injected into the tubule. The rate of injection was controlled to prevent retrograde flow or distension of the tubule. If there were any visible leaks of fluid, any dilation of the tubule or any obstruction to flow, that tubule was abandoned. Distal collection was continued for at least 5 min after the completion of the injection; the collection pipette was then withdrawn and sealed by aspiration of oil from the kidney surface.

The whole of the sample collected was delivered into a counting vial containing P.C.S. scintillant and the two isotopes counted simultaneously with appropriate corrections for dual label counting. Using the same microconstriction pipette replicate determinations of the injected fluid were counted as standards. Samples where recovery of the injected inulin was less than 95% were discarded. Seven pregnant and six virgin animals were used.

Microinjection studies of the collecting ducts

Late distal tubules were identified after a small (50 μl) injection of 10% Lissamine Green into the jugular vein, and when the dye had completely cleared from the kidney the identified tubules were punctured with a microconstruction pipette (as above) which contained 5–10 nl of a solution with NaCl, 70 mmol l^{-1}; KCl, 2 mmol l^{-1}; [^{3}H]inulin, 159 mCi l^{-1}, D-[^{14}C]glucose, 178 mCi l^{-1}. Urine was collected from a bladder catheter (Bishop & Green, 1980) for 16 min after each injection, after which time no inulin was detectable in the urine. Urine was counted for [^{3}H]inulin and [^{14}C]glucose as above and replicate samples of the injectate directly from the constriction pipette diluted with appropriate volumes of 'cold' urine were also counted with corrections for dual label counting and quenching. Again, samples which showed less than 95% recovery of the injected inulin were discarded. Five virgin and five pregnant animals were used.

Results from all experiments are expressed as means ± S.E. of the mean and the significance of any difference was assessed by Student's t test for paired or unpaired data.

RESULTS

As in previous reports (see references in Bishop & Green, 1981) 7–8 day pregnant animals were heavier than virgin controls (243 ± 6 g *vs.* 172 ± 6 g; [$P < 0.001$]) but there was no significant difference in the mean blood pressure which always exceeded 100 mmHg. During the time when micropuncture experiments were performed plasma concentrations of sodium, glucose and potassium remained stable (see Bishop & Green, 1980), and so detailed time courses are not presented; the mean value for each animal during each infusion was calculated from the three or four plasma samples obtained and the mean and S.E. of the mean calculated for both pregnant and virgin animals.

Haematocrit measured during the periods when plasma composition was stable was significantly lower in pregnant animals than in virgins when saline was infused. In both virgin and pregnant animals haematocrit rose significantly during glucose infusion to reach values around 50%; the difference in haematocrit between virgin and pregnant animals during glucose infusion was not statistically significant (Table 1).

Free flow distal collections

Although S.N.G.F.R.s calculated from distal tubular punctures are somewhat different from those previously estimated from proximal tubular punctures (cf Table 1 and Table 1 in Bishop & Green, 1982), there is reason to believe that distal S.N.G.F.R.s are more physiological, in that flow past the macula densa is not interrupted (see Garland & Green, 1982). There were no significant differences in S.N.G.F.R. between pregnant and virgin animals during saline infusion. Changing to glucose infusion made no difference to the S.N.G.F.R. in virgin animals but dramatically increased it in pregnant animals. So, during glucose infusion S.N.G.F.R. in pregnant

TABLE 1. Fluid handling by single nephrons in virgin and pregnant rats during saline and glucose infusions

	Saline			Glucose		
	Virgin	Pregnant	P_1	Virgin	Pregnant	P_2
Haematocrit (%)	46·5 ±0·6	44·3 ±0·8	< 0·05	50·7*** ±1·0	49·3*** ±1·2	n.s.
S.N.G.F.R. (nl min^{-1})	18·47 ±2·10	19·42 ±1·59	n.s.	17·67 ±1·02	23·88 ±1·60**	< 0·01
TF/P_{in}	2·60 ±0·16	2·41 ±0·20	n.s.	3·05 ±0·19	2·64 ±0·13	n.s.
Fluid reabsorption (nl min^{-1})	11·53 ±1·61	10·89 ±1·16	n.s.	11·34 ±0·82	14·35 ±1·23	< 0·05
Number of tubules	21	22		23	24	

P_1 probability that virgin and pregnant animals are different during saline infusion.
P_2 probability that virgins and pregnant animals are different during glucose infusion.
The probability that differences between saline and glucose infusion in either virgins or pregnant animals arose by chance is given *, $P < 0·05$; **, $P < 0·01$; ***, $P < 0·0001$.
n.s. not significantly different.

animals was significantly higher than in virgin controls (Table 1). Since the tubular fluid: plasma (TF/P) inulin ratios were not significantly different in any series, implying that *fractional* reabsorption of fluid was the same, absolute fluid reabsorption was similar in virgin and pregnant animals during saline infusion but during glucose infusion pregnant animals reabsorbed significantly more fluid.

Glucose handling

During saline infusion there was no significant difference in plasma glucose concentration between virgin and pregnant animals; during glucose infusion there was a small difference with virgin animals having a higher plasma glucose concentration than pregnant animals, both values being considerably higher during glucose than during saline infusion (Table 2). These results are similar to those obtained previously (Bishop & Green, 1981).

The concentration of glucose in collected distal tubular fluid was low during saline infusion and was not significantly different between virgin and pregnant animals. During glucose infusion, however, the concentration of glucose in tubular fluid was

considerably higher; virgin animals had a concentration of glucose in tubular fluid similar to that in plasma but pregnant animals had a much lower concentration in tubular fluid than in plasma even though it was still considerably higher than during saline infusion. This lower distal tubular glucose concentration in pregnant as compared to virgin animals during glucose infusion is apparent even when corrected for the differences in plasma glucose concentration in the two series; *TF/P* glucose in virgins was 1·058 and in pregnant animals 0·662. These *TF/P* glucose values are very similar to those found in proximal tubular punctures (c.f. Table 2 with table 2 in Bishop & Green, 1981).

TABLE 2. Glucose handling by single nephrons, up to the site of distal puncture, in free flow experiments

	Saline			Glucose		
	Virgin	Pregnant	P_1	Virgin	Pregnant	P_2
Plasma glucose concentration (mmol l^{-1})	4·76 ±0·26	4·93 ±0·20	n.s.	21·18*** ±1·39	18·11*** ±0·69	< 0·05
Concentration of glucose in distal tubular fluid (mmol l^{-1})	0·53 ±0·07	0·42 ±0·05	n.s.	21·74*** ±0·67	11·93*** ±1·09	< 0·001
TF/P glucose	0·117 ±0·021	0·085 ±0·009	n.s.	1·058*** ±0·018	0·662*** ±0·058	< 0·001
Glucose filtered (pmol min^{-1})	88·6 ±11·6	95·0 ±8·6	n.s.	367·2*** ±21·3	413·0*** ±30·3	n.s.
Glucose reabsorbed (pmol min^{-1})	84·8 ±11·7	91·6 ±5·3	n.s.	224·2*** ±17·8	300·7*** ±25·2	< 0·01
% glucose reabsorbed	94·7 ±0·9	96·5 ±0·5	n.s.	61·5*** ±2·9	73·5*** ±2·6	< 0·01
Number of tubules	21	22		23	24	

For explanation of P_1, P_2 and symbols see Table 1.

The amount of glucose filtered by individual nephrons is similar in virgin and pregnant animals during saline infusion, and although during glucose infusion the filtered load of glucose is dramatically increased there are still no statistically significant differences between virgin and pregnant animals, the higher S.N.G.F.R. in pregnant animals being offset by the lower plasma glucose concentration. During saline infusion most of the filtered glucose was reabsorbed proximal to the distal sites of puncture leaving only a small amount of glucose in the tubular fluid and there were no significant differences between virgin and pregnant animals in the absolute amount or percentage of glucose reabsorbed. During glucose infusion, however, pregnant animals reabsorbed more glucose than virgins in both absolute and relative terms, leaving less in the tubule to be passed on to more distal parts of the nephron.

Sodium handling

There were no statistically significant differences in plasma sodium between the virgin and pregnant animals during either saline or glucose infusion, even though

during glucose infusion the mean sodium concentration was lower than during saline infusion in both groups of animals (Table 3). The concentration of sodium in distal tubular fluid was always lower than in plasma in both virgin and pregnant animals. During saline infusion the sodium concentration in distal tubules expressed either in absolute terms or relative to the plasma concentration was less in pregnant animals; this is consistent with the results presented by Garland & Green (1982). During glucose infusion the concentration of sodium found in the distal tubule was less than that found during saline infusion but the small difference in absolute concentration between virgin and pregnant animals undergoing glucose infusion did not quite reach statistical significance, even though pregnant animals had a significantly lower TF/P sodium.

TABLE 3. Sodium handling by single nephrons, up to the site of distal puncture, in free flow experiments

	Saline			Glucose		
	Virgin	Pregnant	P_1	Virgin	Pregnant	P_2
Plasma Na concentration (mmol l^{-1})	148·9 ±3·1	145·2 ±4·2	n.s.	140·6 ±3·9	142·1 ±2·6	n.s.
Concentration of Na in distal tubule (mmol l^{-1})	75·1 ±6·8	49·1 ±4·3	< 0·01	41·8*** ±2·7	34·2** ±3·1	n.s.
TF/P Na	0·52 ±0·04	0·34 ±0·03	< 0·001	0·30*** ±0·02	0·24** ±0·02	< 0·05
Na filtered (pmol min^{-1})	2746 ±339	2810 ±312	n.s.	2581 ±178	3551 ±258	< 0·01
Na reabsorbed (pmol min^{-1})	2236 ±316	2395 ±293	n.s.	2299 ±170	3222* ±237	< 0·01
% reabsorption of Na	77·7 ±2·6	85·2 ±2·5	< 0·05	88·6*** ±1·34	90·7* ±1·0	n.s.
Number of tubules	21	20		20	22	

For explanation of P_1, P_2 and symbols see Table 1.

Because of the similarities of plasma sodium between the different groups of animals there were no significant differences during saline infusion between virgin and pregnant animals in single nephron filtered load of sodium; during glucose infusion because of the increase in S.N.G.F.R. the filtered load of sodium was significantly higher in the pregnant animals. Similarly the pregnant animals undergoing glucose infusion were the only animals to show an increase in the absolute amount of sodium reabsorbed. Both filtered load and reabsorption of sodium were quite variable however (note S.E. of the means in Table 3), but in general animals which filtered more reabsorbed more sodium. This meant that the percentage reabsorption of sodium by the tubule was much less variable than the absolute reabsorption, and with this reduced variability statistically significant differences became apparent. During saline infusion virgin animals reabsorbed less, proportionately, than did pregnant animals, a finding similar to that described by Garland & Green, (1982). During glucose infusion both virgin and pregnant animals reabsorbed more than during saline

infusion, but the small difference between virgin and pregnant animals was not statistically significant.

Potassium handling

There were no significant differences between the groups of animals in plasma potassium concentrations, tubular fluid potassium concentrations, the filtered or reabsorbed loads of potassium, and so the results are not presented in detail.

TABLE 4. Percentage disappearance of [^{14}C]glucose from tubule after injection

	Saline			Glucose		
	Virgin	Pregnant	P_1	Virgin	Pregnant	P_2
Loop of Henle	94·4 (19) ±0·8	90·7 (21) ±1·2	< 0·02	37·0*** (19) ±4·7	71·3*** (20) ±4·5	< 0·001
Collecting ducts	10·8 (18) ±1·8	5·7 (14) ±1·9	< 0·05	15·2 (20) ±2·0	5·4 (18) ±1·5	< 0·001

For explanation of P_1, P_2 and symbols see Table 1. Numbers in parentheses are numbers of tubules injected.

Microinjection studies

In all the microinjection studies, whether into the loop of Henle or into the collecting duct the amount of [^{3}H]inulin re-collected was not significantly different from 100% of that injected, i.e. there was total re-collection. The percentage of [^{14}C]glucose remaining in the collected fluid was subtracted from 100% and presented as the percentage reabsorption in Table 4. It must be stressed that this represents disappearance of the injected isotope and is not necessarily indicative of *net* glucose transport.

During saline infusion the loop of Henle of pregnant animals reabsorbed less of the injected glucose than did the loop in virgin animals, but in both virgin and pregnant animals the majority of the injected glucose was reabsorbed. During glucose infusion less of the labelled glucose was reabsorbed than during saline infusion, and virgin animals now reabsorbed less than pregnant animals.

In the collecting ducts much less of the injected glucose was reabsorbed and during both saline and glucose infusions pregnant animals reabsorbed significantly less than virgins. It must be emphasized that in these experiments where the fluid was injected into the late distal tubule it was exposed to collecting duct, ureter and bladder epithelium before it was collected.

DISCUSSION

The present experiments were designed to complement ones of a similar nature which have been described earlier (Bishop & Green, 1980; 1981) and to add to our understanding of glucose handling in pregnancy. However, a more recent study on salt and water handling in pregnancy (Garland & Green, 1982) showed some quantitative differences from the present study which require an explanation. The

major difference lies in the absolute magnitude of the S.N.G.F.R.; most other quantitative differences can be explained by reference to this. The S.N.G.F.R. whether measured in proximal tubules (Bishop & Green, 1981) or distal tubules (present data) was very much less than the S.N.G.F.R. reported by Garland & Green (1982). We believe that these differences arise because of the different diets which the animals consumed prior to the experiments. The animals which provided the present data and those for the other studies on glucose handling in pregnancy received a diet containing sodium at a concentration of approximately 0·06 μmol/g^{-1}, whereas the diet of the animals in Garland & Green (1982) contained 0·12 μmol/g^{-1}. It has been shown that animals on a low salt diet have a redistribution of G.F.R. towards the juxtamedullary nephrons and relatively low S.N.G.F.R.s in cortical nephrons; the converse applies for animals on a high salt diet (Horster & Thurau, 1968). It is of interest that the whole kidney G.F.R on the two diets was very similar (cf. Bishop & Green, 1980; Garland & Green, 1982).

In spite of the absolute differences in the S.N.G.F.R. the relative changes in cortical S.N.G.F.R.s are similar to those described by Garland & Green (1982). In both studies there is no significant difference in distal S.N.G.F.R. during saline infusion early in pregnancy. In addition, comparison of the proximal S.N.G.F.R.s reported previously (Bishop & Green, 1981) and the distal S.N.G.F.R.s during saline infusion in this paper reinforces the conclusions drawn earlier (Garland & Green, 1982) about the effects of pregnancy on S.N.G.F.R. and tubuloglomerular feed-back. What is new here is the demonstration of a raised distal S.N.G.F.R. in pregnant animals undergoing glucose infusion. Whether there is a direct effect of glucose on the tubuloglomerular feed-back mechanism is not known and without data in the same nephrons, from the same animals, such a suggestion would be difficult to substantiate. However, there is some evidence that non-electrolytes can have an effect on tubuloglomerular feed-back responses (Bell, Navar, Ploth & McLean, 1980).

The amount of fluid reabsorbed is related to the amount filtered; a constant fraction is reabsorbed, which is similar to that described in the earlier report (Garland & Green, 1982).

Glucose handling

The amount of glucose reabsorbed in the free flow experiments presented in this paper represents the total amount of reabsorption up to the site of the puncture and includes reabsorption by proximal tubules and loops of Henle. In an attempt to quantify the reabsorption in each segment some pertinent calculations are presented in Table 5. There are several underlying assumptions: (*a*) that the percentage reabsorption of glucose in the proximal tubule (Bishop & Green, 1981) is not altered by blockage of the proximal tubule and is the same in the present experiments; (*b*) that glucose is reabsorbed but not metabolized; (*c*) that percentage reabsorption of [^{14}C]glucose represents movement of unlabelled glucose; (*d*) that microinjection techniques did not alter reabsorption. Approximate standard errors of the data have been calculated from the formulae given in Eisenberg & Gage (1969). All standard errors of derived data are large and conclusions must be treated with appropriate reserve. It is convenient to discuss the implications during saline infusion (i.e. with normal plasma glucose concentrations) and during glucose infusion (i.e. with a high plasma glucose concentration) separately.

Normal plasma glucose. At normal plasma glucose concentrations little glucose escapes reabsorption from the proximal tubule and similar amounts are delivered to the loop of Henle. By the time tubular fluid reaches the distal tubule there is still no significant difference between virgins and pregnant animals in the amount of glucose either reabsorbed (Table 2) or remaining in the tubule (Table 5), and no indication that the previously described increase in glucose exretion in pregnant animals (Bishop & Green, 1980) is related to altered function of the loop of Henle or the proximal tubule. Measurements of reabsorption of [^{14}C]glucose indicate that there may be some differences in glucose handling in the loop of Henle but with the small amount of glucose available for reabsorption by the loop in quantitative terms the altered reabsorption is small.

TABLE 5. Calculated reabsorption of glucose in the loop of Henle in virgin and pregnant rats

	Saline		Glucose	
	Virgin	Pregnant	Virgin	Pregnant
% Reabsorption of glucose at end proximal tubule (from Table 2, Bishop & Green, 1981)	83·9 ±0·99	92·8 ±1·03	38·3 ±2·73	58·5 ±3·39
Filtered load of glucose (pmol min^{-1}; from Table 2)	88·6 ±1·6	95·0 ±8·6	367·2 ±21·3	413·0 ±30·3
Glucose remaining at end of proximal tubule (pmol min^{-1}) (*a*)	5·4 ±2·2	6·8 ±2·0	226·6 ±24·3	171·4 ±26·6
Glucose remaining at distal collection site. (pmol min^{-1})	3·7 ±0·6	3·4 ±0·6	143·1 ±14·1	112·3 ±14·1
Net reabsorption by loop of Henle. (pmol min^{-1}) (*b*)	1·7 ±2·2	3·4 ±2·1	83·5 ±28·1	59·1 ±30·1
Reabsorptive flux of glucose (pmol min^{-1}) (*c*)	5·1 ±2·9	6·2 ±2·4	83·8 ±19·9	122·2 ±28·9
Leak of glucose into tubule (pmol min^{-1}) (*d*)	3·4 ±3·6	2·8 ±3·2	0·3 ±34·4	63·1 ±30·6

(*a*) Glucose remaining at end of proximal tubule = (100 − % reabsorption at end of proximal) × filtered load/100.

(*b*) Net reabsorption by loop of Henle = glucose remaining at end of proximal tubule − glucose remaining at distal tubule collection site.

(*c*) Reabsorptive flux of glucose = glucose remaining at end of proximal tubule × percentage reabsorption of [^{14}C]glucose in loop of Henle (see Table 4).

(*d*) Leak of glucose = reabsorptive flux − net reabsorption.

In both virgin and pregnant animals there is reabsorption by the loop of over 90% (Table 4) of the glucose presented and one would expect much smaller amounts of glucose arriving at the distal tubule than were observed (Table 5); the obvious implication is that in the loop of Henle some glucose leaks back into the tubular fluid. Therefore both reabsorption and secretion of glucose occur; this conclusion has also been reached in male rats in the loop of Henle (Bishop *et al.* 1981) and in the proximal tubule (Bishop *et al.* 1978). The calculated leak of glucose was not significantly different in pregnant and virgin animals.

Beyond the distal tubular collection sites there must be reabsorption of glucose in both virgin and pregnant animals and the data from microinjections into late distal tubules indicate that there is indeed uptake from the most distal parts of the urinary system (because urine was collected from the bladder this includes ureter and bladder

as well as collecting ducts) and that the uptake in pregnant animals is much less than in virgin controls (Table 4).

High plasma glucose. At elevated plasma glucose concentrations some of the differences between virgins and pregnant animals are magnified. Similar amounts of glucose are filtered but there is considerably more glucose reabsorbed in pregnant animals prior to the distal puncture site than in virgins (Table 2). Pregnant animals also reabsorbed more glucose in the proximal tubule and passed on less to the loop of Henle (Table 5). Net reabsorption from the loop of Henle was less in pregnant animals (Table 5) even though the reabsorption of [^{14}C]glucose (Table 4) and hence the calculated reabsorption of glucose were higher in pregnant animals (Table 5). The only way these results can be reconciled is by assuming that there is a considerable leak of glucose into the loop of Henle fluid in pregnant animals and very little in virgins (see Table 5). Whether this leak is merely a function of the increased plasma to tubular fluid gradient in pregnancy (see Table 2) or whether it implies some difference in the permeability of the loop of Henle is not known. It is interesting that other substances enter the loop of Henle to a much greater extent in pregnant animals than in virgins (Garland & Green, 1982).

At high and low plasma glucose concentrations, there must be considerable reabsorption beyond the sites of distal puncture and this is indicated by the increased uptake of [^{14}C]glucose in virgins when compared with pregnant animals. Because we have, as yet, no information on the amount of glucose delivered to the collecting duct nor on the differences in response of different populations of nephrons, we can make no comment on the possibility of leakage of glucose into collecting duct fluid.

Considering all the data it seems reasonable that, usually, glucose is predominantly reabsorbed by the proximal tubule with more distal parts of the nephron being able to reabsorb most of the small amount that escapes proximal reabsorption. It is impairment of the net reabsorption of these later segments that occurs in pregnancy; indeed there is evidence to support the view that the proximal reabsorptive sites are actually more efficient in reabsorbing glucose, particularly when the load is increased (Bishop & Green, 1981). Whether the increased loss of glucose in the urine of pregnant animals is primarily a failure of reabsorptive processes or an increased back leak of glucose into tubular fluid is not known.

Sodium handling

The results on sodium handling obtained during saline infusion experiments confirm those previously reported and discussed in detail (Garland & Green, 1982) with a reduction in tubular fluid sodium concentration and an increased percentage reabsorption in pregnant animals. During glucose infusion the concentration of distal tubular sodium was much less than during saline infusion. Bishop *et al.* (1981) have previously shown that perfusion of the loop of Henle with glucose containing solutions increased sodium reabsorption without altering water reabsorption and it is likely that this stimulated mechanism of reabsorption is in the ascending limb of the loop. The small differences between pregnant and virgin animals was of borderline significance ($0{\cdot}1 > P > 0{\cdot}05$ for sodium concentration and $0{\cdot}05 > P > 0{\cdot}02$ for TF/P sodium, Table 3), but much smaller than the differences during saline infusion. Whether this implies that the mechanisms for reducing distal sodium concentration

are the same in pregnancy as during glucose infusion is not known. Whatever the mechanisms involved we were unable to detect a similar effect on potassium.

In pregnant animals during glucose infusion there was an increased filtered sodium which was a consequence of the increase in S.N.G.F.R.; this was associated with an increased reabsorption of sodium. This increased reabsorption does not appear to be due to an increased rate of reabsorption per unit length of proximal tubule (Bishop & Green, 1981) but may be related to the increased length of the proximal tubule in pregnancy (Garland, Green & Moriarty, 1978) or to altered reabsorption in the loop of Henle. The percentage reabsorption was similar in virgin and pregnant animals during glucose infusion and it may be that the increased reabsorption in pregnant animals was merely a consequence of increased tubular flow rate as described for proximal tubules (Green, Moriarty & Giebisch, 1981). The present data is not able to discriminate between these possibilities.

In summary, changes are described in the handling of glucose and sodium by the loop of Henle and also in the handling of glucose by the collecting duct. The altered handling of glucose could explain the increased excretion of glucose during pregnancy in the rat, but the precise mechanisms which are altered have yet to be elucidated.

We would like to thank Mr K. Fletcher for technical support, Miss A. Kaye for secretarial support and the M.R.C. for financial support.

REFERENCES

BELL, P. D., NAVAR, L. G., PLOTH, D. W. & MCLEAN, C. B. (1980). Tubuloglomerular feedback responses during perfusion with non-electrolyte solutions in the rat. *Kidney Int.* **18**, 460–471.

BISHOP, J. H. V. & GREEN, R. (1979*a*). Effects of pregnancy on glucose handling by distal segments of the rat nephron. *J. Physiol.* **289**, 74–75*P*.

BISHOP, J. H. V. & GREEN, R. (1979*b*). Effects of pregnancy on glucose handling by short loops of Henle in the rat. *J. Physiol.* **296**, 91*P*.

BISHOP, J. H. V. & GREEN, R. (1980). Effects of pregnancy on glucose handling by rat kidneys. *J. Physiol.* **307**, 491–502.

BISHOP, J. H. V. & GREEN, R. (1981). Effects of pregnancy on glucose reabsorption by the proximal convoluted tubule in the rat. *J. Physiol.* **319**, 271–285.

BISHOP, J. H. V., GREEN, R. & THOMAS, S. (1978). The effects of glucose on water and sodium reabsorption in the proximal convoluted tubule of rat kidney. *J. Physiol.* **275**, 481–493.

BISHOP, J. H. V., GREEN, R. & THOMAS, S. (1981). Glucose transport by short loops of Henle in the rat. *J. Physiol.* **320**, 127–138.

CHRISTIANSEN, P. J. (1958). Tubular reabsorption of glucose during pregnancy. *Scand. J. clin. Lab. Invest.* **10**, 364–371.

DAVISON, J. M. & HYTTEN, F. E. (1975). The effects of pregnancy on the renal handling of glucose. *Brit. J. Obstet. Gynaecol.* **82**, 374–381.

EISENBERG, R. S. & GAGE, P. W. (1969). Ionic conductances of the surface and transverse tubular membranes of frog sartorius fibres. *J. gen. Physiol.* **53**, 279–297.

FRÖHNERT, P. P., HOHMANN, H., ZWEIBEL, R. & BAUMANN, K. (1970). Free-flow micropuncture studies of glucose transport in the rat nephron. *Pflügers Arch.* **315**, 66–85.

GARLAND, H. O. & GREEN, R. (1982). Micropuncture study of changes in glomerular filtration and ions and water handling by the rat kidney during pregnancy. *J. Physiol.* **329**, 389–409.

GARLAND, H. O., GREEN, R. & MORIARTY, R. J. (1978). Changes in body weight, kidney weight and proximal tubule length during pregnancy in the rat. *Renal Physiol.* **1**, 42–47.

GREEN, R., MORIARTY, R. J. & GIEBISCH, G. (1981). Ionic requirements of proximal tubular fluid reabsorption. Flow dependence of fluid transport. *Kidney Int.* **20**, 580–587.

HORSTER, M. & THURAU, K. (1968). Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. *Pflügers Arch.* **301**, 162–181.

VON BAEYER, H. (1975). Glucose transport in the short loop of Henle of the rat kidney. *Pflügers Arch.* **359**, 317–323.

WELSH, G. W. & SIMS, E. A. H. (1960). The mechanisms of renal glycosuria in pregnancy. *Diabetes* **9**, 363–369.